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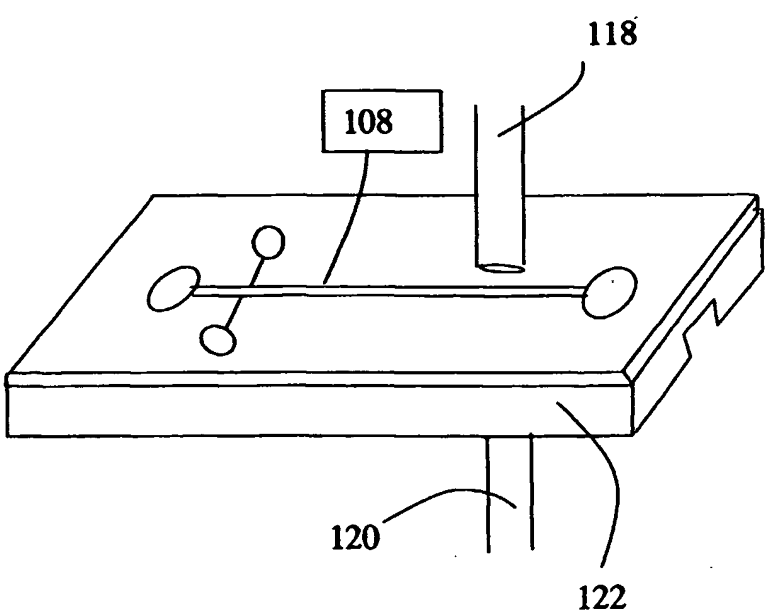
(54) Title: OPTICAL DETECTION SYSTEM FOR CHROMATOGRAPHIC AND ELECTROKINETIC LIQUID PHASE SEPARATIONS



(57) Abstract: An optical system configured to be a detection system simultaneously compatible with conventional separation techniques such as by capillary electrophoresis or micro-HPLC with separations performed using microfluidic devices. The device comprises a light source with an optical fiber (118), a sample holder mount (122) to mount a sample holder in place so that it intercepts light from the light source. There is a detector with a second optical fiber (120) which receives light from the sample. There are means to adjust the spacing between the two fibers (118,120) to adjust the sample space size to accommodate sample holders for the different techniques.

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OPTICAL DETECTION SYSTEM FOR CHROMATOGRAPHIC AND ELECTROKINETIC LIQUID PHASE SEPARATIONS

This application claims the benefit of U.S. provisional application 60/265,414 filed January 31, 2001.

FIELD OF THE INVENTION

This invention relates to an optical detection system for a conventional capillary, and for a microfluidic device used in an analytical separation technique such as capillary electrophoresis (CE), or micro high pressure liquid phase chromatography (micro-HPLC), for both ultra-violet (UV) and light or laser-induced fluorescence (LIF).

Background of the invention

Capillary electrophoresis (CE) or micro high pressure liquid phase chromatography (micro-HPLC) are analytical separation techniques where analytes in a mixture are separated in a capillary. Microfluidic devices are generally planar devices which may perform the separation functions of a CE or micro-HPLC in a sealed microfluidic channel fabricated on a surface of one planar substrate instead of a free-standing capillary. Optical detection systems for these two different forms of separation generally are two different systems.

Microfluidic devices are generally planar devices with at least one surface of which contains interconnecting channels, reservoirs, valves, flow switches, etc., which are fabricated using semiconductor microfabrication technology. This surface is sealed by another planar surface so that liquid moves in enclosed spaces except where samples are injected from the outside world by means of syringes or micropipettes. The channels, etc. are designed to carry out complex laboratory functions such as DNA sequencing. Analytical measurements are also carried out directly on the device. The first of such devices was made of silicon. Since the most convenient method used to transport liquid from one place to another on the device is electrokinetic transport, which employs high voltages in the kilovolt range, most applications in LOC have switched to glass substrates. It is widely believed that

polymer substrates will be the technology that will make LOC truly powerful because of cost and design flexibility considerations.

The advantages of performing the analytical measurements on LOC devices include:

1) integration of laboratory operations, e.g. polymerase chain reaction (PCR) for DNA fragment amplification, to the analytical measurement such as CE or HPLC on the device.

2) Parallel operations of many separations on a single device to increase throughput.

3) Analytical results from a microfluidic format may be superior in quality than those done on conventional bench-top instruments.

SUMMARY OF THE INVENTION

An embodiment of the invention includes an optical detection system for interchangeably analyzing capillary samples and microfluidic device samples. This system comprises a) a light source comprising a first optical fiber; b) a sample holder mount, the sample holder mount adapted to secure a sample holder in a position such that a sample contained by the sample holder intercepts light from the light source; c) a light detector positioned to detect light emitted from the sample, the sample being positioned by the sample holder mount, the light detector comprising a second optical fiber and wherein the light source, the sample holder mount and the light detector are positioned such that a sample space is defined between the light source and light detector; and d) a movable piece adapted to adjust the sample space size.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates a system configuration with a microfluidic device sample positioned between a light source and light detector in the transmission mode.

Figure 2 illustrates the system configuration as in figure 1 during sample transition.

Figure 3 illustrates a system configuration with a capillary sample positioned between a light source and light detector in the transmission mode.

Figure 4 shows two views of a system configuration with a microfluidic device sample positioned between a light source and light detector in the transmission mode.

Figure 5 shows two views of a system configuration with a capillary sample positioned between a light source and light detector in the transmission mode.

Figure 6 shows two views of a system configuration with a capillary sample positioned between a light source and light detector in the transmission mode.

Figure 7 shows two views of a system configuration with a capillary sample positioned between a light source and light detector in the transmission mode.

DETAILED DESCRIPTION OF THE INVENTION

This invention discloses an optical system that is configured to be the detection system for conventional separation techniques such as capillary electrophoresis (CE) or micro-HPLC as well as separation operations using microfluidics, specially a branch of microfluidics commonly known as "lab-on-a-chip (LOC)" or "micro total analytical system (microTAS)".

Thus there are many incentives to transfer the separation functions done on the conventional instrument to the microfluidic device format. However, whereas there are a lot of accumulated studied in the published literature on a variety of methods to separate a large range of mixtures with conventional systems, there are only very few methods in the microfluidic device format because microfluidic devices are a relatively recent development. It is therefore desirable to have a system that can obtain separation results from both the conventional capillary separation and the microfluidic device formats. In this way, all the experimental parameters of the two experiments are the

same except for those pertaining to the capillary and the microfluidic devices themselves. Methods that are originally developed for the capillary can now be more easily and quickly transferred to the microfluidic device format. Also this system will minimize the laboratory space that must be allocated to two different measurement techniques by combining them into one. All instruments in the art performing separation functions as described here are designed specifically to optimize capillary or column separations, or LOC separation, but not both.

This invention discloses an optical detection system comprising a light source emitting light with wavelengths from 190 nm to over 1 micron, an appropriate optical detector for the wavelengths from the light source, optical fibers that couple light to and from the optical source and detector, respectively, a holder which holds a capillary configured to be used in a conventional CE or micro-HPLC separation, or a microfluidic device containing a microfluidic channel configured to perform a CE or HPLC separation. The optical fibers are positioned so that maximum signal to noise ratio is obtained from the light coming from the optical window of the capillary or the microfluidic separation channel in the microfluidic device. The spatial orientation of the optical light source and the optical detector may be in any orientation with respect to the plane of the microfluidic device containing the microfluidic channel performing separation. The preferred orientation of the plane of microfluidic device with open reservoirs is the horizontal position. The preferred orientation of the plane of microfluidics with sealed channels only is any direction with respect to the reference frame of the laboratory.

In the case of the microfluidic device having its plane in the horizontal orientation, the light source and the optical detector are oriented to optimize the signal. In one orientation, the light source and the optical detector may be perpendicular to the plane of the microfluidic device. In another orientation, the light source and the optical detector may be so configured that the light from the light source traverses the longest path possible before reaching the detector. Light source and optical detectors equipped with light-guiding optical fibers to bring the light and the light collecting optics as close to the microfluidic features of interest as possible are preferred.

If a capillary instead of a microfluidic device is used in this system, again the optical light source and the detector are orientated to obtain the best optical signal

through the optical window in the capillary. To facilitate the switching between the capillary and the microfluidic device for this optical detection system, at least one of the optical fibers, either the one from the light source or the one to the detector, may be removed temporarily from its operation position so that the capillary may be easily removed from the optical system, and the microfluidic device inserted, and vice versa. The removal mechanism of the optical fiber may be a hinged mechanism, or any other suitable mechanisms that allow the optical fiber to be put back in its operation position accurately and conveniently.

The optical detection system is part of a larger system that supplies high voltage or pressure to drive fluids, a sample, buffer, and wash and rinse solutions, and waste holders to supply and dispense fluids for the capillary or microfluidic devices, a mechanism for allowing a different fluid to reach the capillary or the microfluidic device at different times, and a control system that allows non-manual control of the events of the whole separation. There are many possible ways to assemble the components in the larger systems. The conventional ways using off-the-shelf components for supplying high voltage or pressure to drive fluids, a sample, buffer, and wash and rinse solutions, and waste holders to supply and dispense fluids for the capillary or microfluidic devices can be assembled by one skilled in the art.

An optical system according to the present invention requires that the only the component holding the sample be changed. All other parts of the system such as the optical source fiber and the optical detector fiber remain at the same locations. Additionally, the optical source fiber, the optical detector fiber and other parts of the system remain in the same locations for optimized operations of both sample measurement techniques. These components may be temporarily removed to facilitate the changing of the capillary to microfluidic device separation operations, and vice versa. Furthermore, the system is configured such that there will be minimal disturbance to the fluids inside the microfluidic devices during the change over from capillary separation to microfluidic separation.

The system according to the present invention also contains peripheral devices so that a separation based on CE or HPLC can be accomplished. Such devices may include a sample, buffer, rinse solutions and waste holders and a mechanism to change their positions in an appropriate sequence so that a CE separation involving

preparing the capillary or microfluidic separation channel can be prepared. In an HPLC separation, all the connections to different mobile phases and pumps to complete the HPLC separation may be included in the device.

To carry out a capillary electrophoresis separation using a microfluidic device according to the present invention using, for example, the configuration of the optical detection system shown in Figure 1, a movable piece 102 holds an optical fiber 104 in the position perpendicular to the sample plane. If the optical fiber is part of the light source, it is focused on the detection window of the microfluidic channel 108. The device frame 100 and the microfluidic device sample holder 110 are designed so that the microfluidic device sample holder can fit in the device frame in only one possible configuration. In this configuration, the optical signal going from the optical source fiber, through the optical window in the microfluidic channel, into the opposite optical detector fiber will be optimized without further adjustment. The movement of the movable piece may be through a hinge mechanism, or the movable piece may be physically removed from the system. The sample holder mount 106 has some guiding or location mechanism built in so that the source optical fiber will be aligned properly to optimize signal detection with the microfluidic device sample holder.

Once the microfluidic device sample holder has been positioned in the device frame and the movable piece has been put in measurement position, the capillary electrophoresis separation on the microfluidic device commences. Buffer and sample injection into the microfluidic separation channel may be accomplished by electrokinetic injection through the application of proper voltages in the sample reservoir and the sample waste reservoir.

Also shown in Figure 1 is a capillary sample 112 in a separation capillary 111 held on a capillary sample holder 114. The capillary sample is inserted into the optical device frame, as shown in Figure 2. The movable piece 102 is opened to allow the capillary sample holder 114 to be inserted on the sample holder mount 106. As shown in this embodiment, a hinge 116 allows the movable piece to adjust the sample holder space.

This optical detection system allows for interchangeably analyzing capillary samples and microfluidic device samples. The sample holder mount is adapted

to secure a sample holder in a position such that the sample contained by the sample holder intercepts light from an optical fiber light source. There is also an optical fiber light detector positioned to detect light emitted from the sample. The light detector optical fiber and the light source optical fiber, along with the sample holder mount define a sample space between the light source and light detector where the samples are positioned. The movable piece adjusts the sample space size to provide the interchangeability of sample types. As such the sample may be either a capillary sample or a microfluidic device sample.

The transition from a microfluidic device sample to a capillary sample is shown sequentially in FIGS. 1-3.

Referring now to FIG. 4, additional details of a microfluidic sample holder 122 are shown. A light source optical fiber 118 and a light detector optical fiber 120 are positioned on either side of a channel 108. The X-X' view of View B illustrates a cutout in the microfluidic sample holder 122 that facilitates collection of high signal to noise optical data by placing the optical fibers proximal to the sample. A microfluidic sample holder 122 may be a self-contained microfluidic device configured as a holder adapted for use with the optical device.

A similar perspective of a capillary sample is shown in FIG. 5, with a capillary sample holder 130 that accommodates a capillary 111 for analytical separation. The capillary sample holder 130 provides for the free ends of the capillary 130 and 124 to pass through the holder, while providing proximal placement of the optical fibers 118, 120.

The optical fibers can also be placed co-planar with the surface of the sample holders. FIG. 6 shows a capillary sample holder 144 that is configured to hold the optical fibers 138, 140 at the upper surface of the sample holder, on either side of the capillary sample 112.

Another configuration for the optical fibers is in a reflection mode, as shown in FIG. 7. The optical fiber 118 emits light towards the sample 112 and the fiber 120 detects the resulting reflected light from the sample.

Examples:

Example 1: A configuration such as that shown in FIG. 1 was used in conjunction with an optical fiber carrying ultraviolet light. The light source was pushed upward (as shown in FIG. 2) on its hinged arm so that the end of the optical fiber would not be hindering the placement of the microfluidic device or the capillary in the holder mount. A polymeric microfluidic device similar in form to the one generally used in analytical separation was analyzed. These devices are typically polymeric devices made of materials such as polydimethylsiloxane or Topas ® which transmit more than 20% of 280 nm light. This microfluidic device was placed in the sample holder mount such that when the optical fiber from the light source was returned to position, the light from the optical fiber was optimized to go through the microfluidic channel, and into the end of the light collector optical fiber placed on the other side of the holder mount. This microfluidic device was used to separate a test sample "A" containing parahydroxybenzoic acid and derivatives obtained from Beckman-Coulter, Inc. for a capillary electrophoresis separation. The microfluidic channel in the device was prepared by a rinsing solution which may be a 0.1 M NaOH aqueous solution. The rinsing solution was pressure injected as known in the art into the microfluidic channel. The conditioning of the channel was completed by repeatedly rinsing with rinsing solution and distilled water as was needed by the experiment. The final step before the separation experiment was to rinse the microfluidic channel with the buffer solution, also supplied by Beckman-Coulter. The buffer solution was a borate buffer with a pH between 8 and 9. A microfluidic channel pre-conditioned with either this rinsing routine or some other means such as a surface coating or treatment optimized for this particular separation, does not require a rinsing routine. To start a separation, the microfluidic channel was first filled with run buffer. A sample injection was accomplished either electrokinetically or with pressure as known in the art. As soon as a sample plug was placed inside the microfluidic channel, a electric voltage was placed in the in buffer reservoir, typically under 5 KV for a total separation channel length of 5 cm, to separate the three components in sample "A" using capillary electrophoresis. An ultraviolet (UV) spectrometer, in this case an optical fiber carrying UV light delivering light to a UV-transmitting part of the microfluidic channel close to the insert of the outlet capillary, and a second optical fiber on the opposite side of the channel so positioned as to receive the optimal amount of UV light passing through the microfluidic channel to the UV light detector, was the detector for the analytes. Three

peaks were recorded as a function of UV absorption around 280 nm against time, which was less than three minutes from the start of the separation to the time when the third peak passed by the optical fiber detector.

At the conclusion of this experiment, the optical fiber from the light source was again pushed upward, and the microfluidic device slid out of the holder mount, and capillary sample was inserted via a capillary sample holder. When the optical fiber light source was put back in place, the set-up was ready for a conventional capillary electrophoresis separation of sample "A". The sample, buffer and waste reservoirs for this experiment are not shown. Using the same set-up, the three peaks of sample "A" were again observed. The results from the two experiments were compared to see whether the microfluidic device had the same performance as the capillary separation device.

Example 2:

The experiment was carried out using an optical fiber light source carrying light from a 488 nm Ar ion laser. The separation sample was a DNA mass ladder containing fragments of 200 base pairs (bp) to 1000 bp in 200 bp intervals. The microfluidic device was filled with a running gel such as hydroxyethylmethylcellulose and a buffer such as EDTA. An intercalating dye that absorbs 488 nm light and fluoresces at 540 nm was also injected into the channel. The mass ladder sample was injected and a run voltage of about 1.5 KV was used. The 5 peaks for the fragments were observed. The microfluidic device was removed as in Example 1 and capillary put in place. The experiment was again carried out as is known in the art for this sample. The results from the two experiments were compared to see whether the microfluidic device had the same performance as the capillary separation device.

What is claimed is:

1. An optical detection system for interchangeably analyzing capillary samples and microfluidic device samples, the system comprising:

a. a light source comprising a first optical fiber;

b. a sample holder mount, the sample holder mount adapted to secure a sample holder in a position such that a sample contained by the sample holder intercepts light from the light source;

c. a light detector positioned to detect light emitted from the sample, the sample being positioned by the sample holder mount, the light detector comprising a second optical fiber and wherein the light source, the sample holder mount and the light detector are positioned such that a sample space is defined between the light source and light detector; and

d. a movable piece adapted to adjust the sample space size.

2. The optical detection system of claim 1 wherein the sample holder comprises a capillary sample.

3. The optical detection system of claim 2 wherein the capillary sample comprises a capillary configured for use in a capillary electrophoresis separation process or a micro-high pressure liquid chromatography separation process.

4. The optical detection system of claim 1 wherein the sample holder contains a microfluidic device sample.

5. The optical detection system of claim 4 wherein the microfluidic device comprises a channel configured for use in a channel separation process.

6. The optical detection system of claim 1 wherein the movable piece is adapted to move the light source.

7. The optical detection system of claim 1 wherein the movable piece is adapted to move the light detector.

8. The optical detection system of claim 1 wherein the movable piece is adapted to move the sample holder mount.

9. The optical detection system of claim 1 wherein the light source emits light with wavelengths from 190 nm to 1 micron.

10. The optical detection system of claim 1 wherein the light detector detects light with wavelengths from 190 nm to 1 micron.

11. The optical detection system of claim 1 wherein the sample holder is adapted to provide openings for the first or second optical fibers.

12. The optical detection system of claim 1 wherein the light detector is positioned to detect reflected light from the sample.

13. A method of spectroscopically analyzing samples, the method comprising:

inserting a first sample holder into a detection device, the first sample holder containing a first sample;

closing the detection device around the first sample holder;

recording a spectroscopic measurement of the first sample;

opening the detection device and removing the first sample holder;

inserting a second sample holder into the detection device, the second sample holder containing a second sample, closing the detection device around the second sample and recording a spectroscopic measurement of the second sample;

wherein the first sample is a capillary sample and the second sample is a microfluidic device sample.

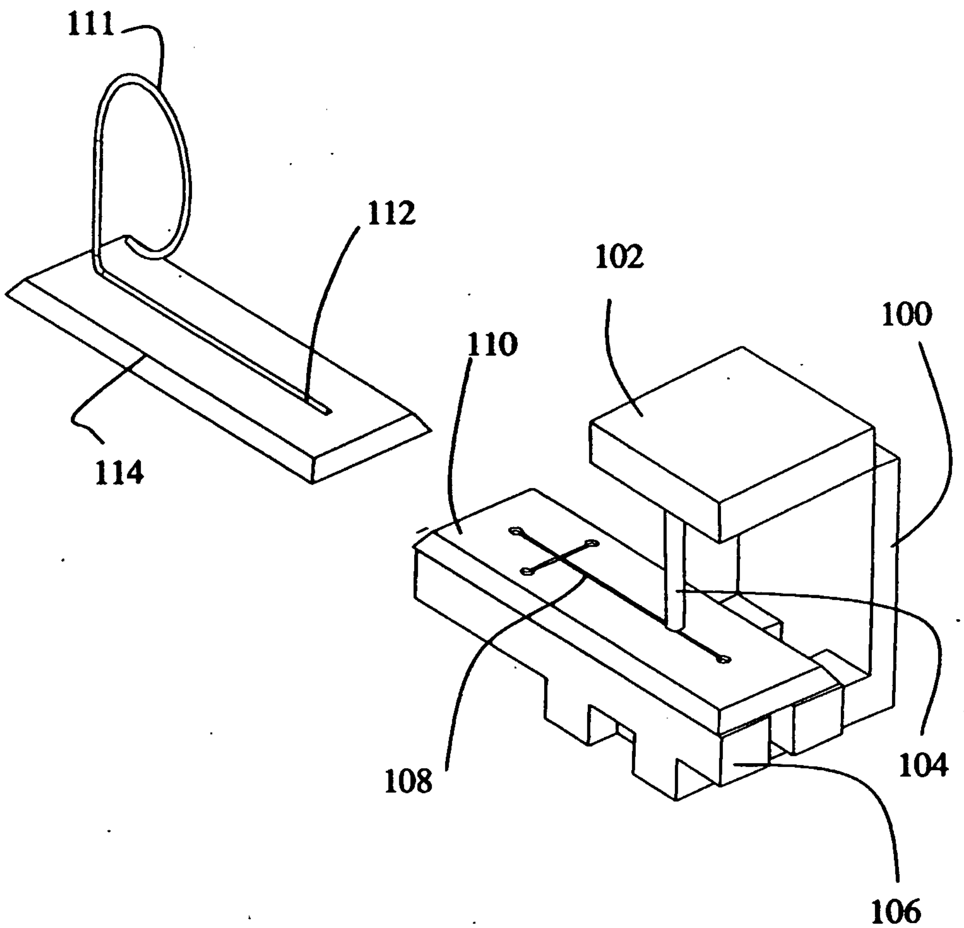

FIG. 1

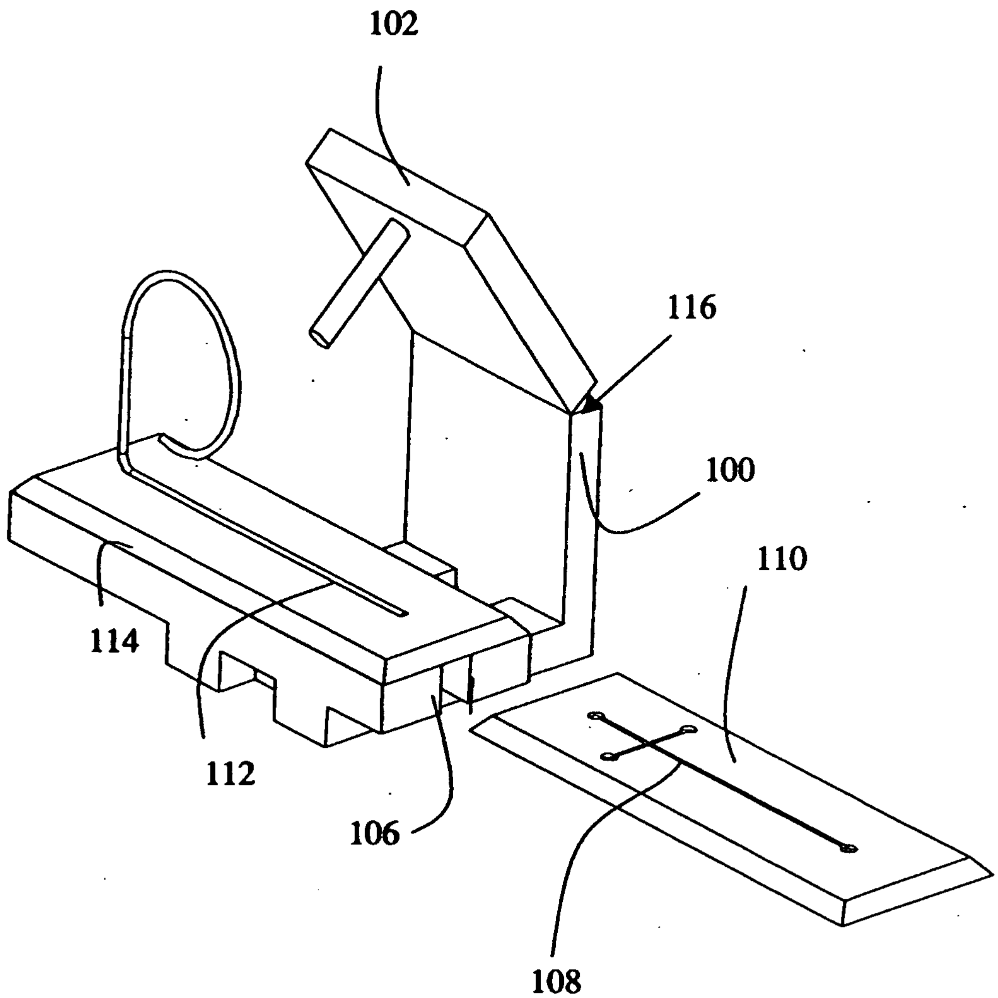

FIG. 2

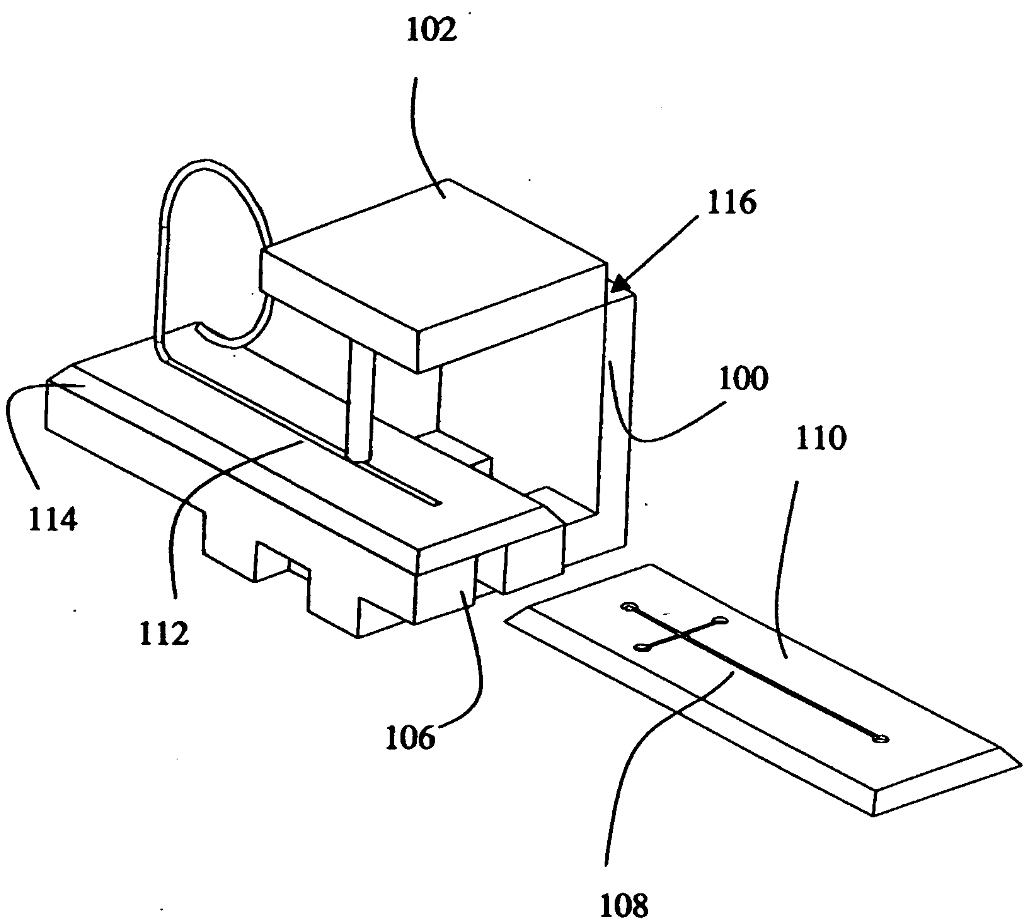

FIG. 3

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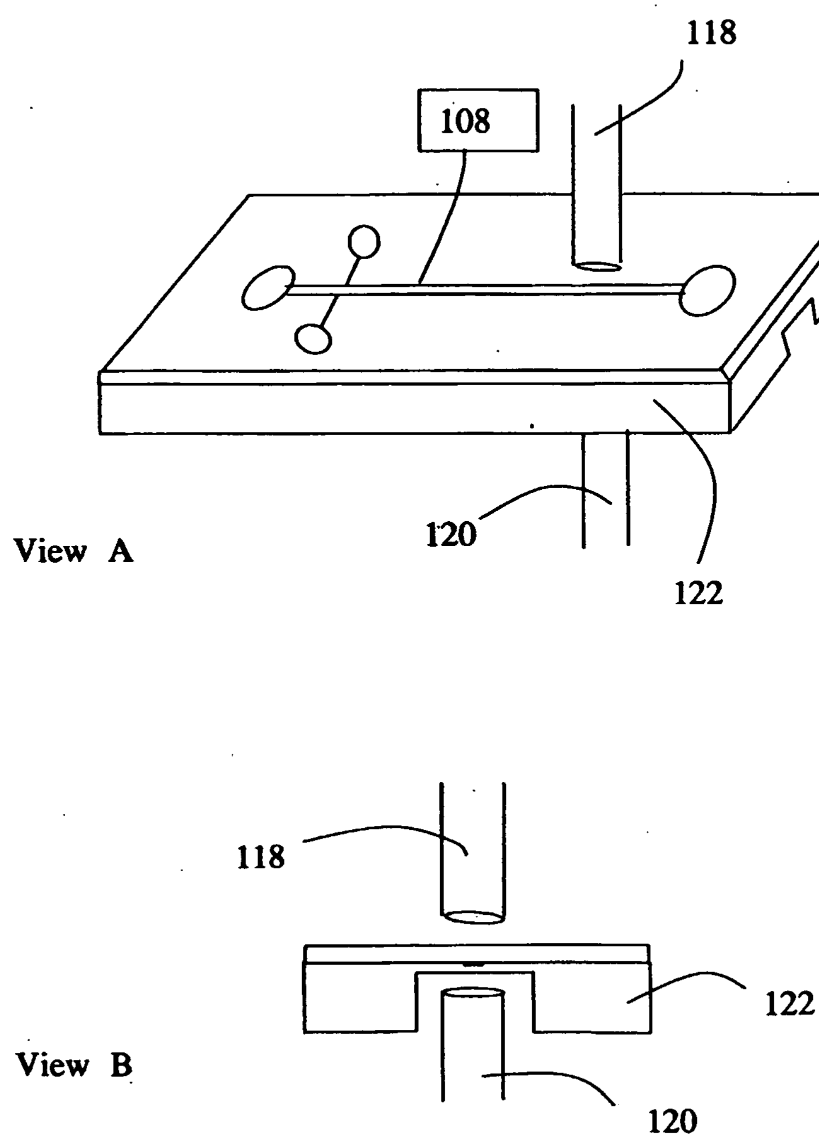
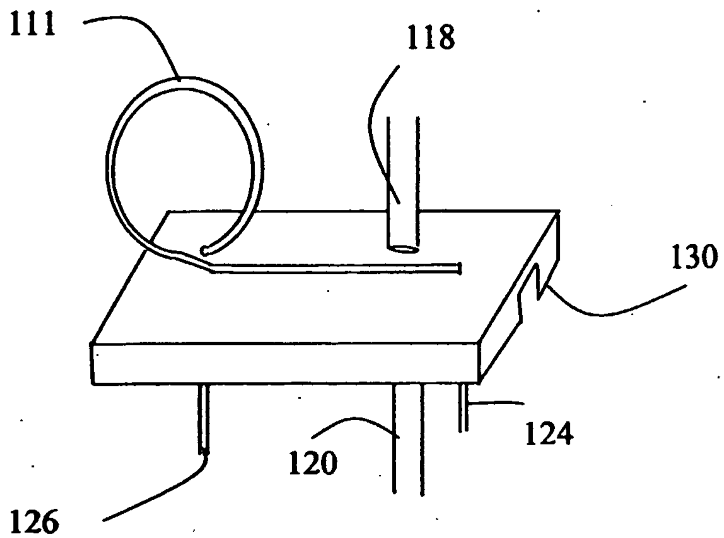


FIG. 4

View A



View B

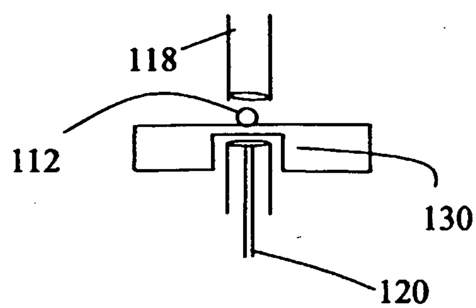


FIG. 5

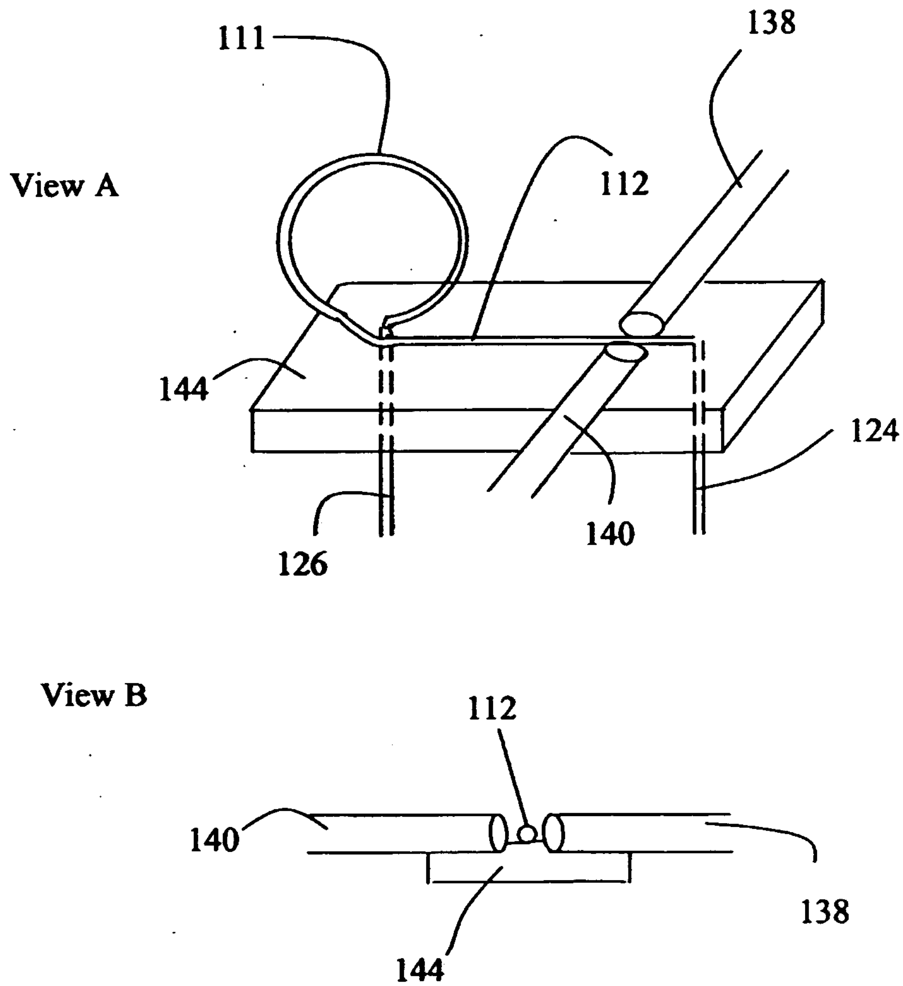
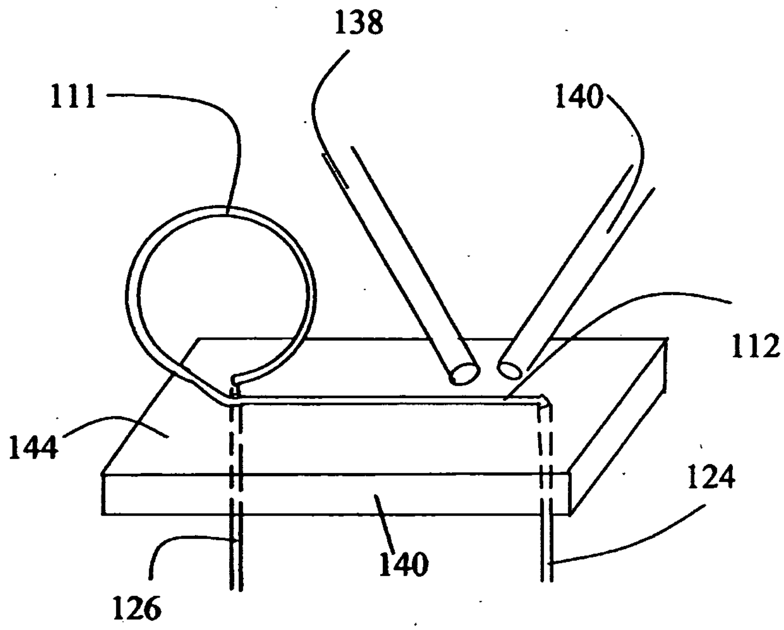


FIG. 6

View A



View B

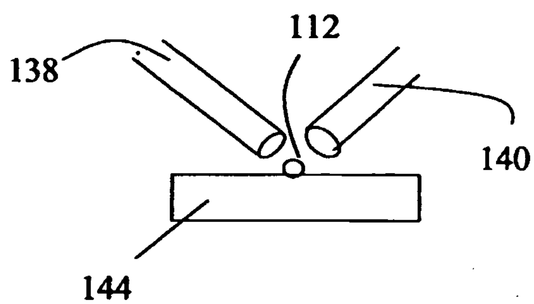


FIG. 7

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/02704

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) :G01N 21/01
US CL :356/244
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 356/244, 246, 344, 440,

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 3,163,176 A 29 December 1964 (29/12/64) see figure 2.	1-13
Y	US 4,312,341 A (ZISSIMOPOULOS et al) 26 January 1982 (26/01/82), see figures 5 and 6.	1-13
Y	US 5,069,552 A (CRAMER et al) 3 December 1991 (03/12/91), see figure 1 and 2	1-13
Y	US 6,069,694 A (VONBARGER) 30 May 2000 (30/05/00), see figure 1	1-13

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
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"O" document referring to an oral disclosure, use, exhibition or other means
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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
04 JUNE 2002	14 JUN 2002

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